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Short communication. Toxicity of emamectin benzoate to adults of *Nesidiocoris tenuis* Reuter, *Macrolophus pygmaeus* (Rambur) (Heteroptera, Miridae) and *Diglyphus isaea* Walker (Hymenoptera, Eulophidae) on tomato plants. Semi-field studies

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Abstract

Whiteflies, the tomato borer and leafminers are among the major pests of greenhouse tomatoes in Spain. *Macrolophus pygmaeus* (Heteroptera, Miridae) and *Nesidiocoris tenuis* are polyphagous predators of whiteflies, the tomato borer and other pests. *Diglyphus isaea* (Hymenoptera, Eulophidae) is an ectoparasite of leafminers. These three natural enemies are commonly released in the commercial horticultural greenhouses of south-eastern Spain. Emamectin benzoate is a new semi-synthetic derivative of the avermectin B_1 developed for Lepidoptera pest control in vegetable crops, with requested inclusion in annex I of the EU directive 91/414/EEC. As for any new insecticide that is being introduced for its use in protected tomato, it is critical to understand the level of compatibility of emamectin in front of those major biological control agents. Potted tomato plants kept in a greenhouse were sprayed with the highest recommended field rate of emamectin benzoate (14.25 mg L^{-1}), and the toxicity of fresh and 7-day-old residues to adults of the three biological control agents were studied to ascertain their compatibility. The results demonstrated the compatibility of both fresh and 7-day-old residues with the three species of natural enemies.

Additional key words: avermectins; biological control; greenhouse; leafminers; whiteflies.

Resumen

Comunicación corta. Toxicidad del benzoato de emamectina sobre adultos de *Nesidiocoris tenuis* Reuter, *Macrolophus pygmaeus* (Rambur) (Heteroptera: Miridae) y *Diglyphus isaea* Walker (Hymenoptera: Eulophidae) en plantas de tomate. Estudios de semi-campo

La mosca blanca, la polilla y el minador de la hoja del tomate son algunas de las plagas más importantes de los invernaderos españoles. *Macrolophus pygmaeus* (Heteroptera: Miridae) y *Nesidiocoris tenuis* son depredadores polífagos de la mosca blanca, la polilla y otras plagas del tomate. *Diglyphus isaea* (Hymenoptera: Eulophidae) es un ectoparasitoide del minador de la hoja. Estos tres enemigos naturales se usan comúnmente en sueltas inoculativas en invernaderos comerciales del sureste español. Benzoato de emamectina es un derivado semi-sintético de la avermectina B_1, desarrollado para el control de plagas de lepidópteros en cultivos hortícolas que está siendo revisado para su inclusión en el Anejo I siguiendo la directiva EU 91/414/EEC. Al igual que para todos los insecticidas de nueva introducción en el cultivo del tomate protegido, es fundamental el conocimiento del nivel de compatibilidad del benzoato de emamectina frente a los agentes de control biológico más utilizados en este ámbito. Se aplicó el insecticida benzoato de emamectina a la dosis máxima recomendada (14,25 mg L^{-1}) sobre plantas de tomate en invernadero para evaluar la toxicidad del residuo fresco y de siete días del insecticida sobre adultos de estos tres agentes de control biológico. Los resultados demuestran que tanto el residuo fresco como el de siete días son compatibles con las tres especies estudiadas.

Palabras clave adicionales: avermectinas; control biológico; invernadero; minador de hojas; mosca blanca.

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Abbreviations used: GABA (gamma-aminobutyric acid), IOBC (International Organization for Biological Control), IPM (integrated pest management).

Spain is an important producer of tomatoes (*Solanum lycopersicum* L.), with a total cultivated area of 62,512 ha in 2009, of which approximately 20,000 ha are protected crops located in the South East (MARM, 2009). Among the key pests of tomato plants in this region are the tobacco whitefly *Bemisia tabaci* (Gennadius) (Homoptera: Aleyrodidae), which is the vector of the *Tomato Yellow Leaf Curl Virus* (one of the most damaging virus diseases of tomato) (Gabarra *et al*., 2006; Calvo *et al.,* 2008), and the tomato borer *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), which was recently introduced in the country (Urbaneja *et al.*, 2009). Moreover, other pests such as leafminers of the genus *Liriomyza,* the thrips *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), spidermites and other Lepidoptera can threaten the agricultural production of fresh market tomatoes (Robledo-Camacho *et al.,* 2009).

The generalist predators *Nesidiocoris tenuis* Reuter (Heteroptera: Miridae) and *Macrolophus pygmaeus* (Rambur) (Heteroptera: Miridae) (previously known as *M. caliginosus* Wagner; Martínez-Cascales *et al.*, 2006) prey on whiteflies, leafminers, spider mites and several Lepidoptera species including *T. absoluta* (Urbaneja *et al.*, 2009). These predators are regularly released in the greenhouses of south-eastern Spain (Van der Blom, 2002; Calvo and Urbaneja, 2004; Urbaneja *et al.*, 2005; Robledo-Camacho *et al.*, 2009). Similarly, *Diglyphus isaea* (Walker) (Hymenoptera: Eulophidae), a solitary and endemic ectoparasitoid of *Liriomyza* species (Diptera: Agromyzidae) in the Mediterranean area (Chow and Heinz, 2005), is also released in the tomato crop at the beginning of the growing season (Van der Blom, 2002; Robledo-Camacho *et al.*, 2009). All of these natural enemies of tomato pests are included in the official list of commercialised beneficial control organisms in Spain (MARM, 2010a), as required by agricultural regulations.

During the last years, most tomato growers in the Spanish Mediterranean region have been using almost exclusively biological control within Integrated Pest management (IPM) programs. Due to the severity of the crop damage produced by the tomato borer, the arrival of this new pest has unbalanced the ecosystem and revealed the need to find new alternative control measures, including natural enemies and selective insecticides. In this sense the introduction of any new selective pesticide in tomato production must be integrated with biological control organisms but also with the practice of bumblebee pollination.

Emamectin benzoate (4”-deoxy-4”-methylamino-4”-epiavermectin B_1 benzoate) is a new macrocyclic lactone insecticide derived from the avermectin family of natural products. These products have been developed for the control of Lepidoptera pests on a variety of vegetable crops worldwide (Liguori *et al.*, 2008), with a particular efficacy against *T. absoluta*. The compound is not systemic, but it exhibits good translaminar activity (Willis and Mc Dowell, 1989). Its main physiological mode of action is to stimulate the release of the γ-aminobutyric acid (GABA) neurotransmitter, thus causing a continuous flow of chloride ions into the muscle cells resulting in a suppression of contraction and paralysis (Ishaaya *et al*., 2002). Emamectin acts basically via ingestion and, to a lesser extent, by direct contact (Dybas and Babu, 1988), and it is rapidly metabolised by photodegradation (Ishaaya *et al*., 2002). This insecticide is currently being evaluated for inclusion in Annex I of the European directive 91/414/EEC which regulates the registration of plant protection products, and for its potential use in Spain (MARM, 2010b).

The purpose of our study was to test the compatibility of emamectin with adults of the three selected natural enemies under semi-field conditions.

All experiments were conducted in an experimental glass greenhouse located in Madrid (Spain) equipped with cooling and heating systems, during February-July 2008, under controlled environmental conditions (25 ± 5°C; 40 ± 5% RH). Tomato plants cv Montfavet 63/5 HF1 (Vilmorin) were seeded in tray cells with sterilized peat substrate (pH 6.5) and transplanted into individual pots (22 cm in diameter for *N. tenuis* and *M. pygmaeus*, and 12 cm in diameter for *D. isaea*) four or six weeks later (6 weeks for *N. tenuis* and *M. pymaeus*, and 4 weeks for *D. isaea*) to obtain homogeneous pesticide-free plants. Each plant (of about 20 cm height for *N. tenuis* and *M. pygmaeus*, and 10 cm for *D. isaea*) was surrounded by a plastic cylinder (21 cm in diameter, 30 cm in height, for *N. tenuis* and *M. pygmaeus*, and 10.5 cm in diameter, 12 cm in height, for *D. isaea*) sealed to the surface of the pot with foam and covered with a fine mesh to allow a proper ventilation. *N. tenuis* was supplied by Koppert Biological Systems S.L. (Mirical®, Águilas, Murcia, Spain) and *M. pygmaeus* and *D. isaea* by Syngenta Bioline S.A. (Macroline® and Digline® respectively, Aguadulce, Almería, Spain). For each insect species and trial, eight tomato plants were sprayed to the point of run-off with 14.25 mg a.i. L^{-1} (highest recommended field rate) of emamectin benzoate (Affirm®, 0.95 SG, Syngenta Agro S.A., Madrid) and eight with distilled water as a control. Once the residues dried, 15 adults of the test species were released per pot cage. In the case of the predators (*M. pygmaeus* and *N. tenuis*), eggs of *Ephestia kuenhiella* Zeller (Lepidoptera: Pyralidae), supplied by Koppert Biological Systems S.L. (Entofood®, Aguilas, Murcia, Spain), were added *ad libitum* to the tomato leaves as food. *D. isaea* adults were fed by introducing a water-honey mix. Mortality was recorded at 24 h, 48 h and 72 h after treatment. To evaluate the effect of 7-day-old residues, one week after the treatment, 15 new adults were released per pot on the same treated plants from the fresh residue trial, and mortality was evaluated as reported above.

Mortality data [mean values and standard errors (SE)] from each type of bioassay and insect species were analysed by ANOVA followed by a least significant difference (LSD) test at the 95% confidence level using Statgraphics® Plus, version 5.0 (STSC, 1987). The insecticide was classified into the four International Organisation for Biological Control (IOBC) standard classes for semi-field tests using the following criteria (Hassan, 1994): 1 = harmless (< 25% corrected mortality), 2 = slightly harmful (25-50% corrected mortality), 3 = moderately harmful (51-75% corrected mortality) and 4 = harmful (> 75% corrected mortality).

The percentage of mortality of *N. tenuis, M. pygmaeus* and *D. isaea* after exposure to fresh and 7-day-old residues of emamectin is shown in Table 1. Both fresh and 7-day-old residues of emamectin benzoate did not significantly increase the mortality of the predatory species *N. tenuis* or *M. pygmaeus* compared to controls (IOBC class 1). In the case of the parasitoid *D. isaea*, however, three days of contact killed significantly more adults (35.83%) compared to the control (14.16%). The increase in mortality (25.2%) slightly surpassed the upper limit of the IOBC toxicity rating 1 for semi-field trials, and thus, the insecticide was classified as IOBC class 2. After the same period of contact with 7-day-old residues, however, no differences in mortality were found in either treated or control units (14.99% and 15.80% respectively), and the insecticide was classified as IOBC class 1.

Our results show that emamectin is harmless for adults of *N. tenuis* and *M. caliginosus* when exposed to fresh

Table 1. Percentage of cumulative mortality of adults of three natural enemies exposed to fresh and 7-day-old residues of 14.25 mg a.i. L^{-1} emamectin benzoate in greenhouse tomato plants

Treatment	Fresh residues			7-day-old residues		
	24 h	**48 h**	**72 h**	**24 h**	**48 h**	**72 h**
Nesidiocoris tenuis						
Control	7.75 ± 2.74	8.58 ± 3.09	11.08 ± 3.35	8.45 ± 3.57	9.74 ± 3.74	9.74 ± 3.74
Emamectin benzoate	10.25 ± 4.74	11.92 ± 4.51	12.75 ± 4.45	5.89 ± 1.19	8.11 ± 2.65	8.11 ± 2.65
df	1;14	1;14	1;14	1;10	1;10	1;10
F-value	0.21	0.37	0.09	0.46	0.12	0.12
p-value	0.655	0.552	0.769	0.512	0.731	0.731
Macrolophus pygmaeus						
Control	1.67 ± 1.67	2.49 ± 1.75	2.49 ± 1.75	0.00 ± 0.00	0.00 ± 0.00	2.22 ± 2.22
Emamectin benzoate	1.67 ± 1.09	2.49 ± 1.21	2.49 ± 1.21	1.11 ± 1.11	1.11 ± 1.11	1.11 ± 1.11
df	1;14	1;14	1;14	1;10	1;10	1;10
F-value	0.00	0.00	0.00	1.00	1.00	0.20
p-value	0.999	0.999	0.999	0.341	0.341	0.664
Diglyphus isaea						
Control	2.49 ± 1.21	7.49 ± 1.96	14.16 ± 2.65[a]	2.49 ± 1.21	6.66 ± 2.18	15.83 ± 4.35
Emamectin benzoate	2.49 ± 1.75	12.91 ± 2.30	35.83 ± 3.31[b]	2.49 ± 1.75	14.99 ± 4.31	14.99 ± 2.09
df	1;14	1;14	1;14	1;10	1; 10	1; 10
F-value	0.00	3.19	25.99	0.00	2.97	0.20
p-value	0.999	0.096	> 0.001	0.999	0.107	0.854

Within species and columns, data followed by different letters are significantly different ($p < 0.05$; ANOVA and LSD mean comparison).

residues. These results are in line with those found by Tedeschi *et al.* (2002) on *M. pygmaeus* nymphs using a topical application of emamectin at a dose of 15 mg a.i. L^{-1} in a laboratory study. Similarly, no deleterious effects have been reported in other predatory species, such as the larvae of *Chrysoperla externa* (Hagen) (Neuroptera: Chrysopidae) or the mites *Euseius citrifolius* (Denmark and Muma) (Acari: Phytoseiidae) and *Euseius alatus* (DeLeon) (Acari: Phytoseiidae) (Reis *et al*., 2004; Ferreira *et al*., 2006). In contrast with these results, Studebaker and Kring (2003) reported that the predatory bug *Orius insidiosus* (Say) (Heteroptera: Anthocoridae) was very sensitive to fresh residues of emamectin in semi-field conditions using cotton plants. Even though the type of substrate used in the testing procedures might affect the results, particularly if using plant substrates, due to the differences in leaf hairiness, cuticular waxes, leaf veins, etc. (reviewed in Croft, 1990), the toxicity of emamectin to this predatory insect was similar in the laboratory using an inert substrate and in semi-field and field conditions using cotton plants (Studebaker and Kring, 2003).

The lack of harmful effects of emamectin by residual contact in many natural enemies of plant predators might be expected because the insecticide primarily acts via ingestion (Dybas and Babu, 1988). In contrast with the selectivity reported for most predatory species, in other studies, fresh residues of this insecticide seem to negatively affect some species of parasitoids belonging to the order Hymenoptera when assessed in laboratory but not in semi-field studies with fresh or aged residues. As such, under laboratory conditions, fresh residues of 10 mg a.i. L^{-1} in cabbage leaves were very harmful to *Aphidius gifuensis* Ashmead (Braconidae) and *Cotesia plutellae* Kurd (Braconidae) females, causing 91% and 94% mortality, respectively, after 24 h of contact (Haseeb and Amano, 2002; Kobori and Amano, 2004). Additionally, fresh residues (17 g a.i. ha^{-1}) in celery leaves caused 75% mortality in *D. isaea* adults within the first thirty minutes after treatment (Chukwudebe *et al.*, 1997). The higher mortality recorded by Chukwudebe *et al*. (1997) with fresh residues in extended laboratory conditions in comparison to our semi-field assays could be explained by the differences in substrate and size of the arena (detached single leaf *vs.* a whole plant in our trial).

The fast degradation on the plant surface of the residues of emamectin benzoate is crucial for their selectivity (Prabhu *et al*., 1991). In accordance to this fast degradation on the surface, our semi-field assays with *D. isaea* and tomato plants showed IOBC toxicity ratings for fresh and 7-day-old-residues of 2 and 1, respectively, and in those assays with the two predators, class 1. Similarly, Chukwudebe *et al.* (1997), who investigated this parasitoid on celery plants, also noted a rapid decrease of mortality with an increase in residue age (50% mortality after exposure to 3.5 hours old residues of 17 g a.i. ha^{-1} compared to 20% when they were between 35 and 60 hours old). The time elapsed to achieve residue harmlessness is dependent on whether the study was carried out under semi-field or laboratory conditions. When the exposure to emamectin residues took place on leaves deposited in Petri dishes in the laboratory, the insecticide at 10 mg L^{-1} had a longer toxic effect and was harmful for *A. gifuensis* and *C. plutellae* adults for up to 14 and 17 days, respectively, after exposure (Haseeb and Amano, 2002; Kobori and Amano, 2004). Several factors seem to be implicated in the harmlessness of aged residues under greenhouse compared to laboratory conditions. The most important factor is probably the faster photodegradation of the insecticide on the leaf surface that could happen in the greenhouse during the first hours after application compared to the effect of artificial light on detached leaves kept in Petri dishes in the laboratory (Jansson *et al*., 1997). A quick degradation and a faster absorption and translocation of emamectin diminish the amount of insecticide deposited on the plant surface and lower the possibility of poisoning the natural enemies due to contact with the insecticide, while the fast penetration provides enough amount of the product into the leaf tissues to ensure an effective control of plant feeding pests (Prabhu *et al*., 1991). Another factor to be taken into account is the greater ability of insects to avoid contact with pesticide deposits through increased mobility and successful location of shelters on whole plants compared to detached leaves.

In conclusion, emamectin benzoate applied under greenhouse conditions at a dose of 14.25 mg a.i. L^{-1} immediately before releasing *M. pygmaeus, N. tenuis* and *D. isaea* was compatible with their continued survival. Thus, we can recommend its use for IPM programs in Spanish tomato greenhouses.

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